Diatomaceous Earth Increases the Efficacy of *Beauveria bassiana* Against *Tribolium castaneum* Larvae and Increases Conidia Attachment

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ABSTRACT This research tested the suppressive ability of *Beauveria bassiana* (Balsamo) Vuillemin alone and in combination with diatomaceous earth against the red flour beetle, *Tribolium castaneum* (Herbst). Adults did not show a dose response to *B. bassiana*, and the addition of diatomaceous earth (DE) did not result in a significant increase in mortality. Against larvae, however, DE at 190 mg/kg grain enhanced the efficacy of *B. bassiana* at all concentrations ranging from 33 to 2,700 mg of conidia per kilogram of grain. The presence of DE resulted in 17- and 16-fold decreases in the median lethal concentration of *B. bassiana* at 56 and 75% RH, respectively. No significant differences in larval mortality in response to *B. bassiana* and diatomaceous earth alone or in combination were found between 56 and 75% RH. Conidial attachment to larvae was significantly greater with 190 mg/kg DE than without it. The partial analysis of lipids taken up by DE from the larvae revealed the removal of phospholipids and long-chain fatty acids. These results support the hypothesis that diatomaceous earth enhances the efficacy of *B. bassiana* against larval *T. castaneum*, at least in part by damaging the insect cuticle, thus increasing conidial attachment and making nutrients more available to conidia for their germination.

KEY WORDS *Tribolium castaneum*, *Beauveria bassiana*, diatomaceous earth, synergism, relative humidity

Beauveria bassiana (BALSAMO) Vuillemin is a well-known entomopathogen with a broad host range and is regarded as a safe biopesticide (Anonymous 2000). Probably the greatest factor in the loss of inoculum viability of entomopathogenic fungi under field conditions is inactivation caused by UV light (Ignoffo and Garcia 1992, Braga et al. 2001). Most grain storage and processing environments do not have that disadvantage. Many of the important pests in these environments have proven to be susceptible targets for *B. bassiana*, but its relatively high production costs and high application rates render it economically impractical (Hluchy and Samsinakova 1989, Adane et al. 1996, Rice and Cogburn 1999, Bourassa et al. 2001, Lord 2001, Meikle et al. 2001, Padin et al. 2002).

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan insect pest of storage facilities and processing plants. Widespread resistance to malathion, resmethrin, and bioresmethrin has been reported in this species (Haliscak and Beeman 1983, Beeman and Wright 1990, Zettler and Cuperus 1990, Arthur 1992). Although *B. bassiana* has been reported as a naturally occurring pathogen of the red flour beetle in Great Britain and Kenya (Burges and Weiser 1973, Oduor et al. 2000), it is not highly pathogenic for the beetles (Padin et al. 1997, 2002). High doses of *B. bassiana* are also required for other stored grain pests (Hluchy and Samsinakova 1989, Moino et al. 1998, Rice and Cogburn, 1999, Meikle et al. 2001), and it may not be attractive for many real-world applications. Thus, there is a need to establish use rates that would be commercially acceptable for the stored grain environment. The efficacy of *B. bassiana* can be increased in different ways. Rice and Cogburn (1999) suggested that the abrasive nature of rice hulls might be responsible for the greater efficacy of *B. bassiana* against lesser grain borer and rice weevil on rough rice than on nonabrasive media. On this basis, they recommended the development of abrasive conidial formulations of *B. bassiana.*

Because *B. bassiana* must adhere to, germinate on, and penetrate through the host integument, we hypothesized that its efficacy may be improved in the presence of other surface-active agents. Diatomaceous earth (DE) is one such agent that has been reported to cause abrasion and adsorption of the cuticular lipids (Ebeling 1971). Lord (2001) found DE, amorphous silicon dioxide, and diamond dusts to have a synergistic interaction with *B. bassiana* against some

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stored grain pests, while showing no negative effects on germination of the fungus.

Entomopathogenic fungi such as *B. bassiana* are usually considered to be more effective at higher relative humidity, but reports differ on the importance of ambient humidity for the infection of *B. bassiana* in various insects (Ferron 1977, Ramoska 1984, Searle and Doberski 1984, Marcandier and Khachatourians 1987, Luz and Fargues 1999, Haraprasad et al. 2001). Because DE is a desiccant, it is most effective at low humidity under which insects transpire at a high rate (Mewis and Ulrich 2001a). Diatomaceous earth and *B. bassiana* seem to be complementary in their moisture optima.

Only adults have been used in previously reported studies on the interaction of *B. bassiana* and red flour beetles. Our objectives in these studies were to evaluate the interaction between *B. bassiana* and DE when combined against the red flour beetle and to explore the mechanisms underlying their interaction. We tested adults as well as larval stages and found that adults were highly tolerant to *B. bassiana* isolate that we used and, therefore, chose larvae for further studies. Specifically, we assessed the degree of conidial attachment in the presence and absence of DE and detected subcuticular exudate. We also evaluated the effects of two relative humidities on the efficacy of *B. bassiana* and DE, alone and in combination, because of the varying reports on the effects of relative humidity on the efficacy of *B. bassiana.*

Materials and Methods

Fungus, DE, and Insects. All of the experiments were conducted at the USDA Grain Marketing and Production Research Center, Manhattan, KS, with *T. castaneum* from a laboratory colony that has been maintained since 1958 and has an eastern Kansas origin. Specimens of *T. castaneum* have been placed in the Kansas State University Museum of Entomological and Prairie Arthropod Research, Department of Entomology, Kansas State University, Manhattan, KS, under voucher number 148. Commercially produced conidia of *B. bassiana* strain GHA (Emerald BioAgriculture, Butte, MT), DE formulated with 10% silica gel (Protect-It, Hedley Technologies, Blaine, WA), and hard red winter wheat with a moisture content of 11.3% were used in all experiments. The *B. bassiana* technical powder contained 9.4×10^{10} conidia per gram. To check for viability, the conidia were spread on Sabouraud dextrose agar with a cotton swab and incubated for 18 h at 26°C. Germination rates were at least 90% throughout the study.

Effect of *B. bassiana* and DE on Adults. Adults were exposed to *B. bassiana* concentrations ranging from 500 to 2,000 mg/kg. The treatments were mixed thoroughly with the grain by manually shaking the test tubes. Ten grams of the treated grain were transferred to 30-ml plastic cups. Twenty adults of mixed ages and sexes were placed into the grain in each cup. Each treatment and untreated controls had three replications per experiment, and each experiment was repeated at least three times with different generations of insects. The experimental units were placed in individual boxes with a saturated sodium chloride solution, resulting in a relative humidity of 75 ± 1%. The boxes were then placed in a walk-in environmental chamber at 26 ± 1°C. After 8 d, adult mortality was recorded. In a separate experiment, the adults were treated with 500-2000 mg/kg *B. bassiana* together with 1000 mg/kg DE. Because red flour beetle adults experienced very low mortality in the initial experiments, no further tests were performed on this life stage.

Effects of DE and *B. bassiana* on Larvae at Two Different Relative Humidities. Diatomaceous earth at 63, 125, 190, and 250 mg/kg and *B. bassiana* at 125, 250, 500, and 1000 mg/kg were tested individually against larvae by treating crimped wheat as described above. To evaluate whether there was an interaction between the two agents, *B. bassiana* at 33–2,700 mg/kg concentrations was tested with and without a fixed concentration of DE (190 mg/kg). We chose 190 mg/kg DE because it caused <30% mortality when tested alone. Twenty 10-d-old larvae (from the date of oviposition) were placed in cups that contained 10 g of treated wheat. Each treatment and the untreated control had three replications per experiment, and each experiment was repeated at least three times with different generations of insects. To determine the effect of relative humidity on the efficacy of *B. bassiana* and DE against red flour beetle larvae, the abovementioned experimental units were incubated at two relative humidities, 75 ± 1 and 56 ± 2%. We selected these relative humidities as representative of the environmental conditions frequented by stored grain pests (Reed et al. 1989), including red flour beetle (Shazali and Smith 1986). Plastic boxes with saturated solutions of sodium chloride and sodium bromide were used to provide the higher and lower relative humidities, respectively. HOBO data recorders (Onset Computer, Pocasset, MA) were used to record the temperature and relative humidity. Temperature was maintained at 26 ± 1°C in all boxes. For each relative humidity, there were three different boxes in a split-plot design where humidity was the main treatment and concentration was the subtreatment. The boxes were placed in a walk-in environmental chamber, and larval mortality was recorded after 8 d.

Effect of DE on Conidial Attachment. To determine whether DE affects the attachment of *B. bassiana* to red flour beetle larval cuticle, conidia were stained with fluorescein isothiocyanate (FITC) according to Lord (2001). Ten grams of crimped wheat was treated with 1000 mg/kg FITC-labeled conidia, with or without 190 mg/kg DE. Thirty 10-d-old larvae were placed in each treatment and incubated at 75 ± 1% RH overnight. Conidia attached to 50 insects for each treatment were counted after washing with 0.1% Silwet L-77 (Loveland Industries, Greeley, CO). The entire ventral and dorsal surfaces of larvae were scored for conidia attachment at 400× under fluorescent illumination with an excitation wavelength of 460–490 nm, chromatic mirror at 505 nm and barrier filter of 515–

Table 1. Mortality of *T. castaneum* adults at various concentrations of *B. bassiana* with and without 1000 mg/kg DE after 8 d at 75 ± 1% RH

DE	*B. bassiana* (mg/kg)				
	0	500	1000	1,500	2,000
			% Mortality (SD)		
−	0a	6.1 (5.4)aA	10.0 (5.0)aA	6.1 (6.0)aA	8.3 (2.5)aA
+	4.4 (1.9)b	7.2 (6.6)aA	6.1 (4.1)aA	5.5 (6.3)aA	7.2 (5.0)aA

Each value based on average of three experiments, 300 insects per experiment, and three replicates per treatment per experiment. Means within columns followed by same lowercase letters and means within rows followed by same uppercase letters do not differ significantly from each other at $P > 0.05$ (Fisher's LSD test).

550 nm. There were three replications of these treatments done on three separate days.

Lipid Detection. A partial analysis of the lipids taken up by DE was carried out to help clarify its effects on surface components of beetle larvae. After 2–3-h confinement of beetle larvae with 200 mg of DE, the dust was removed from treated larvae by vigorously shaking them in sieves. Lipids were extracted from DE that had not been applied to larvae, DE that was removed from larvae, and from untreated larvae. The lipids were extracted using chloroform:methanol (2:1) solution after Bligh and Dyer (1959), concentrated under a nitrogen stream, and separated by spotting 10 μl of each sample on thin-layer chromatography (TLC) plates by using hexane, diethyl ether, and acetic acid (80:20:1) as the solvent. Neutral lipids (Sigma, St. Louis, MO) were run as standards. The TLC plates were placed in an iodine chamber to visualize the spots. Phospholipids were identified by spraying molybdenum blue reagent (Sigma), whereas fatty acids absorbed by DE were analyzed by gas chromatography (GC)-mass spectrometry (McCloskey 1970).

Data Analysis. The adult and larval mortality responses to treatments and the conidial attachment data passed normality testing and were not transformed. They were analyzed using PROC GLM (SAS Institute 2002). The interaction between DE and *B. bassiana* was evaluated by χ^2 analysis of expected and observed mortalities as described by Lord (2001). The LC_{50} values were calculated with Polo-PC software (LeOra Software 1987). The effect of relative humidity on the efficacy of two agents alone or in combination was also analyzed using PROC MIXED (SAS Institute 2002), and the mean mortalities at each of the concentrations between the two relative humidities were compared by Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

Results

Effect of *B. bassiana* and DE on Adults. There was no dose response of the adults to *B. bassiana*, and at concentrations of 500 and 2,000 mg/kg only 6.1 ± 5.4 and 8.3 ± 2.5% mortalities were obtained, respectively. The addition of 1000 mg/kg DE to all the tested concentrations of *B. bassiana* did not increase mortality of the adults. There was no mortality in the control of any experiment (Table 1).

Effect of DE on Larval Mortality at Two Different Relative Humidities. Larval mortality ranged from 7.7% to a maximum of 60% over the range of tested concentrations. No significant differences in the response of red flour beetle larval mortality were detected between 56 ± 2 and 75 ± 1% RH ($F = 0.62$; df = 1, 4; $P = 0.48$) (Table 2). However, there were highly significant differences in larval mortality resulting from different concentrations of DE ($F = 116.91$; df = 4, 76; $P < 0.0001$). There was no mortality in the untreated control. At 56 ± 2% RH, the LC_{50} value of DE was 248.9 mg/kg (95% CI, 215.1–309.5 mg/kg), whereas at 75 ± 1% RH, the LC_{50} value was 298.3 mg/kg (95% CI, 243.8–423.0 mg/kg).

Effect of *B. bassiana* on Larvae at Two Relative Humidities With and Without DE. *T. castaneum* larvae, unlike the adults, showed a highly significant dose response to *B. bassiana* ($F = 140.7$; df = 5, 92; $P < 0.0001$). Mortality at the lowest concentration of 33 mg/kg was 7.2 and 10% at 56 ± 2 and 75 ± 1% RH, respectively. Mortality with 2,700 mg/kg *B. bassiana* was 62.2 and 62.7% at 56 ± 2 and 75 ± 1% RH, respectively. There was no mortality in the controls.

Table 2. Mortality of *T. castaneum* larvae at various concentrations of DE and two relative humidities

RH (%)	DE (mg/kg)				
	0	63	125	190	250
			% Mortality (SD)		
56	0	7.7 (7.9)aA	17.7 (12.5)aB	27.2 (7.9)aC	60.0 (8.2)aD
75	0	9.4 (5.8)aA	13.3 (9.6)aAB	23.3 (9.3)aB	53.3 (16.2)aC

Each value based on average of three experiments, 300 insects per experiment, and three replicates per treatment per experiment. Means within columns followed by same lowercase letters and means within rows followed by same uppercase letters do not differ significantly from each other at $P > 0.05$ (Fisher's LSD test).

Table 3. Mortality of *T. castaneum* larvae at various concentrations of *B. bassiana* and two relative humidities

RH (%)	*B. bassiana* (mg/kg)					
	0	33	100	300	900	2700
			% Mortality (SD)			
56	0	7.2 (5.6)aA	15.5 (7.2)aB	26.1 (9.2)aC	38.8 (12.9)aD	62.2 (9.7)aE
75	0	10.0 (7.9)aA	15.5 (6.3)aA	29.4 (11.0)aB	40.0 (12.9)aC	62.7 (7.9)aD

Each value based on average of three experiments, 300 insects per experiment, and three replicates per treatment per experiment. Means within columns followed by same lowercase letters and means within rows followed by same uppercase letters do not differ significantly from each other at $P > 0.05$ (Fisher's LSD test).

There was no significant interaction between *B. bassiana* efficacy and relative humidity ($F = 0.14$; df = 5, 92; $P = 0.98$), and the mean mortalities at each of the concentrations tested did not differ significantly from each other between the two relative humidities ($P > 0.05$) (Table 3). However, larval mortality was significantly higher under all test concentrations of *B. bassiana* with 190 mg/kg DE than without it ($P < 0.0001$) (Tables 4 and 5). Chi-square values >3.84 indicate synergism of DE for all the tested concentrations of *B. bassiana* both at 56 ± 1% (Table 4) and 75 ± 1% RH (Table 5). There was no mortality in the untreated controls either at 56 ± 2 or 75 ± 1% RH.

The LC_{50} value of *B. bassiana* alone was 1463.1 mg/kg (1107–2065 mg/kg) for red flour beetle larvae at 56 ± 2% RH, whereas the addition of 190 mg/kg DE resulted in an LC_{50} value of 84.8 mg/kg (95% CI, 57–117 mg/kg). The log-probit regression lines had slopes of 0.90 without DE and 0.78 with DE at 56 ± 2% RH. At 75 ± 1% RH, the LC_{50} value for *B. bassiana* in the presence of DE was 86.8 mg/kg (95% CI, 62–115 mg/kg), in contrast to an LC_{50} of 1,370.6 mg/kg (95% CI, 1012–2002 mg/kg) for *B. bassiana* alone. The regression slopes at 75 ± 1% RH were 0.84 without DE and 0.81 with DE. A comparison of efficacy of *B. bassiana* with DE showed no significant differences between the two relative humidities tested at any of the concentrations ($P > 0.05$).

Attachment of *B. bassiana* Conidia. The number of *B. bassiana* conidia attached to larval cuticle was significantly greater with DE present than without it ($t = 5.34$, df = 2, $P = 0.033$). Most of the conidia were observed around the intersegmental membrane regions of the insect body. However, conidia were denser on thoracic segments (dorsal as well as ventral sides) of the insect body than on the abdomen. The mean counts of conidia were 212.7 (95% CI, 131.6–343.7) with DE, and 90.92 (95% CI, 56.26–146.93) without DE. We were unable to detect germinated conidia because the staining treatment did not penetrate into the germ tube.

Lipid Detection. A partial analysis of the lipids present in the DE that was removed from the insects indicated the presence of long-chain fatty acids and phospholipids. The TLC plates with extracts of the untreated larvae or of the DE that was removed from insects consistently developed only two stained areas with our method. One of the spots remained at the origin and stained blue with molybdenum blue reagent, indicating the absorption of phospholipids from the insects by DE. No phospholipids were detected in the untreated DE extract. The composition of other stained region ($R_f = 0.15$) from the extracts was identified as long-chain free fatty acid by comparison with an oleic acid standard. The GC-mass spectrometry analysis confirmed the presence of long-chain fatty acids.

Discussion

Both adults and larvae of the red flour beetle proved to be very tolerant to *B. bassiana* and DE when either was tested alone. The adults were especially tolerant and did not show a dose response to *B. bassiana.* High-dose requirements for the adults of *T. castaneum* have also been reported by Rice and Cogburn (1999)

Table 4. Chi-square analysis based on expected and observed mortalities of *T. castaneum* larvae due to *B. bassiana* and 190 mg/kg DE at 56 ± 2% RH and 26°C

Treatment Bb (mg/kg)	Expected mortality (%) (if DE is additive)	Observed mortality (%) (±SD[a])	χ^2
0		23.3 (5.0)	
33	28.3	40.5 (11.0)	7.3
100	34.5	52.2 (9.7)	13.8
300	43.0	62.2 (13.0)	15.0
900	52.2	75.5 (9.1)	21.7
2700	70.7	92.2 (7.9)	22.3

Bb, *Beauveria bassiana.*
Significant χ^2 for synergism is >3.84 at $P > 0.05$.
[a] Standard deviation for three trial means.

Table 5. Chi-square analysis based on expected and observed mortalities of *T. castaneum* larvae due to *B. bassiana* and 190 mg/kg DE at 75 ± 1% RH and 26°C

Treatment Bb (mg/kg)	Expected mortality (%) (if DE is additive)	Observed mortality (%) (±SD[a])	χ^2
0		19.4 (0.98)	
33	27.1	38.8 (9.6)	6.9
100	31.1	51.1 (7.4)	18.6
300	42.4	65.5 (8.0)	21.8
900	51.4	76.1 (11.6)	24.4
2700	69.2	91.6 (3.5)	23.5

Bb, *Beauveria bassiana.*
Significant χ^2 for synergism is >3.84.
[a] Standard deviation for three trial means.

and Padin et al. (2002). However, even at a lower concentration of 300 mg/kg, *B. bassiana* has been shown to have a comparatively better performance on other stored grain pests such as *Rhizopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), and *Cryptolestes ferrugineus* (Stephens) (Lord 2001). Similarly, high DE dose requirements for the red flour beetle adults have been reported in past studies (Korunic 1998, Mewis and Ulrich 2001b, Rigaux et al. 2001). The high doses of *B. bassiana* and DE used in those studies do not seem to be practical for commercial application of these agents.

We know of no prior reports on the efficacy of *B. bassiana* against larval stages of the red flour beetle. In our studies, the larvae showed a dose-dependent mortality, but no significant differences were observed in the efficacy of *B. bassiana* against larvae at the two relative humidities tested. Ambient humidity has been found to have little impact on *B. bassiana* efficacy in some insect systems (Moore 1973, Ferron 1977). Ramoska (1984) reported that *B. bassiana* conidia were infective against the chinch bug, *Blissus leucopterus* (Say), over a broad range of relative humidities. Marcandier and Khachatourians (1987) also reported no significant effects of relative humidity on *B. bassiana*-related mortality of grasshoppers at relative humidities ranging from 12 to 100%. The preceding reports and our results differ from those of Huafeng et al. (1998) and Luz and Fargues (1999), both of whom reported significant effects of humidity on germination and infection rates of *B. bassiana.* A significant increase in the mortality of coffee berry borer, *Hypothenemus hampei* (Ferrai), due to *B. bassiana* was reported with an increase in relative humidity from 50 to 90% (Haraprasad et al. 2001). Luz et al. (1998) screened isolates of *B. bassiana* for the control of *Triatoma infestans* (Klug) and reported varying effects of 50 and 100% RH on the efficacy of different isolates. These differences in the effects of relative humidity on *B. bassiana* efficacy may have occurred because of differences in the microclimate on the cuticle or possibly from differences in cuticular chemical composition of different hosts.

In our experiments, DE alone was not an effective control agent for *T. castaneum* larvae after 8 d of treatment. However, Subramanyam et al. (1998) reported 96% mortality of first instars of *T. castaneum* after 21 d when another formulation, Insecto, was applied to shelled maize at the rate of 125 and 250 mg/kg. The differences in the DE formulation, instar, and duration of exposure may explain these differences in larval response. We did not find significant differences in DE-related larval mortality for the two relative humidities that we tested. However, reported activity of DE against adult *S. granarius* (Mewis and Ulrich 2001a) and *O. surinamensis* (Arthur 2001) and larval *Ephestia kuehniella* (Zeller) (Nielsen 1998) decreased with increased relative humidity. We used larvae in these studies, but adult *T. castaneum* have been used in past studies to determine the effects of relative humidity on the efficacy of dusts. Arthur (2000a) reported a decrease in the efficacy of DE against red flour beetle adults as relative humidity increased from 40 to 75% when insects were deprived of food. Aldryhim (1990) also observed a decrease in activity of silica dust toward *Tribolium confusum* (Du Val) adults with an increase in relative humidity from 40 to 60%. Some of the differences in the abovementioned results may be due to differences in the chemical and physical properties of DE used (Quarles 1992), life stage tested, or presence of food. Food may decrease the efficacy of DE, even at lower relative humidity, by enabling the beetles to produce metabolic water that neutralizes the effects of DE (Quarles 1992, Arthur 2000b, Mewis and Ulrich 2001a).

B. bassiana interacts differently with different supplemental agents. For example, diflubenzuron has been found to be additive (Delgado et al. 1999), and imidacloprid has been reported to be antagonistic (James and Elzen 2001) or synergistic (Quintela and McCoy 1997, 1998a,b; Furlong and Groden 2001) with *B. bassiana.* In the current study, we used a nontoxic grain protectant, DE, and found that at 190 mg/kg, it had a synergistic interaction with all tested concentrations of *B. bassiana* and at both tested relative humidities. Our findings coincide with those of Lord (2001) who reported a synergistic interaction between DE at 200 mg/kg and *B. bassiana* in the range of 11–300 mg/kg against *R. dominica* and *O. surinamensis.* In contrast, Brinkman and Gardner (2001) found no significant increase in the mortality of the fire ant *Solenopsis invicta* (Buren) when they combined DE with *B. bassiana.* Our results on increased conidial attachment in the presence of DE are not in agreement to those of Lord (2001), who found no significant effect in the case of *R. dominica.* Differences in results may be related to differences in the cuticular composition of these insect species.

We observed conidial attachment to the larval cuticle around all intersegmental regions. However, the maximum density of conidia was on dorsal and ventral surfaces of the thoracic segments with no apparent association with integumentary processes. These observations contrast with those of some previous studies in which maximum conidial attachment occurred on body regions with a greater number of setae or spines (Boucias et al. 1988, Sosa-Gomez et al. 1997). However, this is not consistent, because Fernandez et al. (2001) found the legs of *L. decemlineata* to be the region where most of *B. bassiana* conidia attached. Quintela and McCoy (1998b) reported generally uniform attachment of conidia of *M. anisoplae* on the cuticle of larval *Diaprepes abbreviatus* L., but fewer conidia were attached to setae and setal sockets. The differences in placement of conidia attachment in the aforementioned cases suggest that topology of insect cuticle may play an important role in fungal attachment.

Lord (2001) observed a stronger interaction of *B. bassiana* with absorbent silica dust than with abrasive diamond dust and proposed that lipid removal may contribute more to the synergistic interaction between *B. bassiana* and DE than abrasion. Mewis and Ulrich (2001a) also stated that the principal effect of

DE is sorptive and not abrasive. However, abrasion of the cuticle along with absorption of cuticular waxes has also been argued as a mode of action of DE (Golob 1997, Korunic 1998). The enhanced effect of combining *B. bassiana* and DE, contrasted with their ineffectiveness as single factors, is interesting. We observed an increase in the number of conidia attached to the cuticle and removal of lipids in the presence of DE. The limited cuticular damage due to DE alone at 190 mg/kg caused <30% mortality, but absorption of cuticular lipids and release of subcuticular compounds correlates with increased conidial attachment and increased mortality.

Cuticular lipids act as a barrier to the loss of water from the insect's body and protect insects from pathogenic microorganisms. For example, fatty acids with 10 or less carbons have fungistatic and fungicidal properties (Koidsumi 1957, Smith and Grula 1981, Saito and Aoki 1983). However, long-chain fatty acids that are usually predominant in insect cuticle (Blomquist and Dillwith 1985) have been reported as having no negative effects on the germination of fungi. In fact, they may even accelerate fungal growth (Smith and Grula 1981, Smith and Grula 1982, Saito and Aoki 1983, Bidochka and Khachatourians 1992). Within the limits of our partial analysis, we identified long-chain fatty acids and an area that stained positively for phospholipids in the only discernible TLC spots obtained from DE that was removed from *T. castaneum* larvae. Other lipids may also be present that were not detected by our method. Phospholipids generally are not considered to be a part of insect cuticle but rather are embedded in the internal membranes (Gibbs 1998). Apparently, damage to the cuticle due to the abrasive, and perhaps the absorptive, properties of DE may have caused the release of subcuticular compounds that had a positive effect on the pathogenic fungus. The exact mechanisms by which DE interacts with *B. bassiana* are not clear but may involve a combination of increased availability of water and other nutrients, removal or mitigation of inhibitory materials, alteration of adhesive properties, and physical disruption of the cuticular barrier.

The use of *B. bassiana* alone for the control of red flour beetle does not seem to be a promising option because of the high application rates required. However, addition of within-labeled-rate dose of DE increases its efficacy significantly. The synergism of DE with *B. bassiana* observed in the current study, and by Lord (2001), affirms this combination to be a good candidate for inclusion in large scale testing for integrated pest management programs for the red flour beetle control. Furthermore, the stable, UV-free nature of the storage environment, and the efficacy of *B. bassiana* at operative ambient humidities give support to its development for commercial applications, particularly where conventional chemical agents cannot be used. Although the economic feasibility of this combination remains to be established, the data presented here help to move cost and efficacy toward that goal for a particularly recalcitrant pest.

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